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Effect of contaminated fish meal stress on histology of rat (*Rattus norvegicus*) ovary

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Abstract

Study was undertaken to elucidate the effect of contaminated fish meal diet on the ovary of albino rat *Rattus norvegicus*. Histological parameters of 7 and 30 days control and experimental rats were evaluated to assess the impact of contaminated fish meal stress on ovarian histology. The present study revealed healthy follicles to significantly decrease with significant increase in atretic follicles in *treated rats*

Keywords: Histological parameters, atretic follicles

Introduction

Ovary is the most significant site of toxic damage affecting fertility of the female reproductive system. A variety of substances are known to be ovarian toxins Hirshfield *et al.*, (1991) [9], Plowchalk, *et al.*, (1993) [11], Cooper *et al.*, (1993) [4], Adilaxmamma *et al.*, (1994) [1], Ratansooriya *et al.*, (1995) [13] and Anajulia *et al.*, (2006) [2]. Hirshfield *et al.*, (1997) [8] have reported toxicants to destroy primordial follicles resulting in depletion of non-renewable follicles and hence leading to permanent infertility. Goldman *et al.*, (1994) [4] and Baligar *et al.*, (2001) [3] have reported pesticide toxicity to inhibit secretion of FSH and LH hormones, thus affecting folliculogenesis and ovulation in rats. Pesticides and heavy metals cause ovarian atrophy by arresting oogenesis Kaliwal *et al.*, (2001) [12] and Anajulia. 2006) [2].

Although extensive cytomorphological studies has been made on the toxicity of various agents on ovary, little is known about the histology. A review of the literature indicates that there is not much information on the effect of contaminated fish meal diet on the female reproductive system of rats. Hence, the present study was undertaken to elucidate the effect of contaminated fish meal diet on the ovary of albino rat *Rattus norvegicus*.

Materials and Methods

Habitat Selection: Yellamallappa Lake, which is around 400 acres, located in Aavalahalli, near Hoskote, Bangalore north was selected for study (Figures 1 and 2).



Fig : 1



Fig : 2

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Experimental animal: Thirty days old female albino rats weighing about 50 grams were selected randomly from the breeding stock, housed in clean separate plastic cages, bedded with paddy husk, covered with chrome plated grills and maintained under uniform husbandry conditions of light and temperature.

Feed Preparation: Adult fish *Tilapia mossambicus* were obtained from Yellamallappa tank. About 25 fishes weighing about 110 to 150 grams were used for feed preparation. Fishes were dried in sunlight and powdered to uniform size particles. Powder was weighed accurately and suspended in distilled water and then made into pellets using pelletizer. Pellets were sun dried for 3- 4 days, stored in air tight containers and used as feed for experimental rats.

Maintenance in The Laboratory: Rats were randomly segregated into four groups, two groups served as control and other two as experimental groups. Control groups were fed with standard rat feed (gold mohar) procured from Hindustan Lever Limited, and given water ad libitum. The experimental groups were fed with contaminated fish meal and maintained in the laboratory for a period of 7 and 30 days.

Fixation and Sectioning of Tissue: The ovary was fixed in Bouin's fluid for 24 hours, dehydrated in alcohol and embedded in paraffin wax. 6µ thick sections were cut and stained with haematoxylin and eosin for histopathological studies.

Histological Evaluation: Stained sections of the ovary were examined under the light microscope. The general histological appearance was assessed. Follicles were classified and described according to the method given by Briefly, follicles are classified as primordial when they have an intact oocyte with a single layer of squamous epithelium, primary if they have a oocyte with a single layer of cuboidal granulosa cells, preantral if they contained an oocyte and 2-4 layers of granulosa cells with no antral space, antral if they contained three or more layers of granulosa cells and a clearly defined antral space, atretic if they contain apoptotic granulosa cells (defined by the appearance of apoptotic bodies in the granulosa cell layers), disorganized granulosa cells and a degenerating oocyte.

Results and Observation

1. 7 days control and treated rat ovary

Control rat ovary contained large number of follicles in early developmental stages. The ovary of experimental animals however, contained fewer number of developing follicles and many atretic follicles (Fig: 3 & 4).

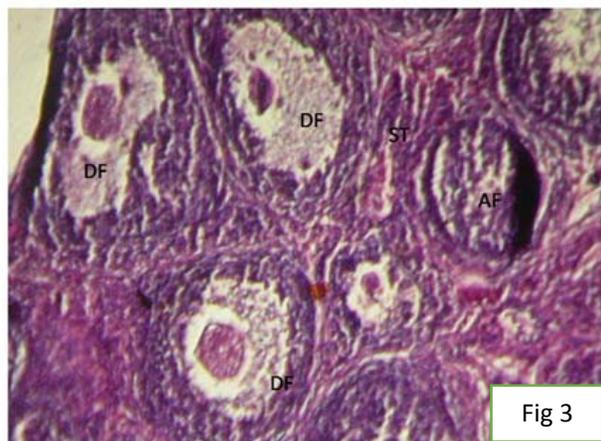


Fig 3: Photomicrograph of paraffin section of control ovary of rat, *R. norvegicus*, for 7 days showing the presence of large number of early developing follicle

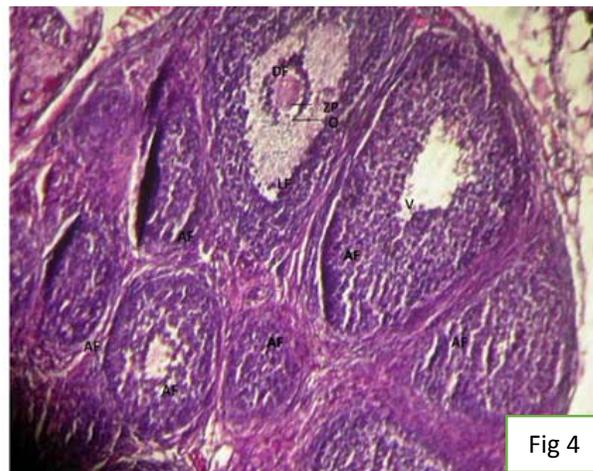


Fig 4: Photomicrograph of paraffin section of experimental ovary of rat, *R. norvegicus*, fed with contaminated fish meal diet for 7 days showing plenty of atretic follicle and developing follicle

2. 30 days control and treated rat ovary

Control rat ovary contained fewer atretic follicles and follicles in various developmental stages (primary, secondary and graafian) (Fig.5). Healthy follicles of control rat had a centrally placed oocyte with intact zona pellucida. Microvilli from the oocyte and thicker prolongations from granulosa cells were seen to penetrate the zona pellucida. Antrum was beginning to develop and contained little liquor folliculi (Fig: 6.). Some of the secondary follicles had a crescent shaped antrum with liquor folliculi. It was seen pushing the oocyte away from the centre. Membrana granulosa was compact, Cumulus oophorus was beginning to be formed. Theca externa has well-organized intact spindle shaped cells (Fig: 7). The Graafian follicle is larger in size and the oocyte eccentrically placed. The follicle is surrounded by cumulus oophorus. Dicus proligerus is seen connecting the oocyte to wall of the follicle. Antrum is well developed, crescent shaped and filled completely with liquor folliculi. Stroma is highly vascularised (Fig: 8)

In 30 day treated rats, the ovary was observed to have large number of atretic follicles, fewer number of unhealthy follicles and an irregular germinal epithelium. (Fig: 9). No healthy follicles were present. Some secondary follicles are vacuolated and have no oocyte. The stroma contains large spaces and dilated blood vessels. Granulosa cells shows less staining intensity. (Fig: 10). In some of the developing follicles there is no connection between the oocyte and the granulosa cells. Some secondary follicle show indications of antral development. Theca externa is formed as an intermediate layer between oocyte and antrum, thereby pushing the oocyte towards the follicular wall depicting abnormality (Fig: 11). In some early graafian follicles, the follicle contains thin theca externa. Antrum is disorganized but filled with liquor folliculi. Zona pellucida is disintegrated with distorted oocyte. Stroma contains reduced blood vessels (Fig: 12). The graafian follicle had distorted oocyte and irregular shaped antrum. The antrum did not have liquor folliculi. Theca externa was thin. The cells of the membrana granulosa showed pycnosis (Fig: 13 & 14).

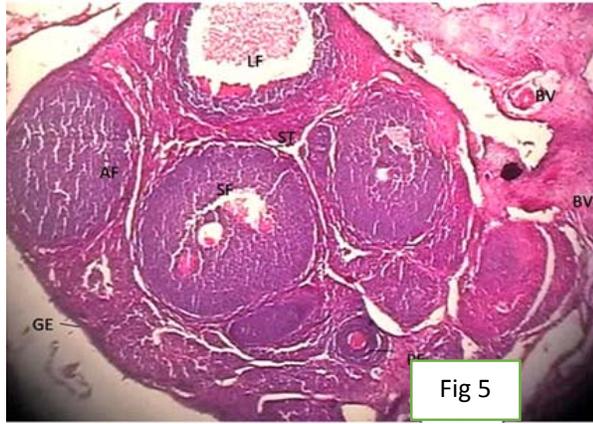


Fig 5

Fig 5: Photomicrograph of paraffin section of control ovary of rat, *Rattus norvegicus*, showing the presence of follicles in various developmental stages and atretic follicle

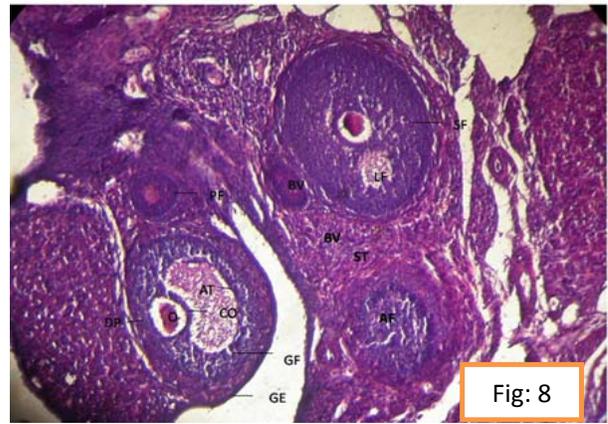


Fig: 8

Fig 8: Photomicrograph of paraffin section of control ovary of rat, *R. norvegicus*, shows graafian follicle with crescent shaped antrum filled with liquor folliculi and stroma is highly vascularised.

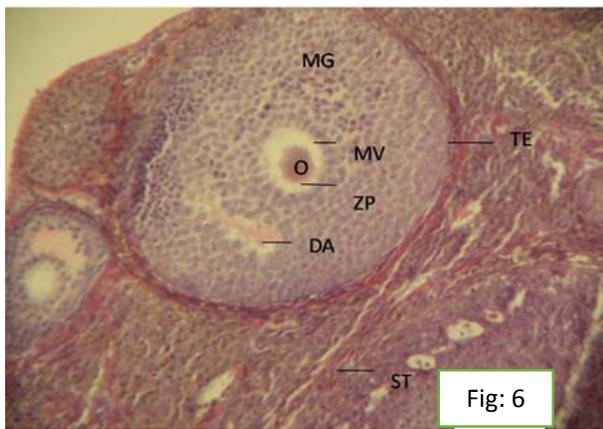


Fig: 6

Fig 6: Photomicrograph of paraffin section of control ovary of rat, *Rattus norvegicus*, depicting secondary follicles with centrally placed oocyte with intact zona pellucida

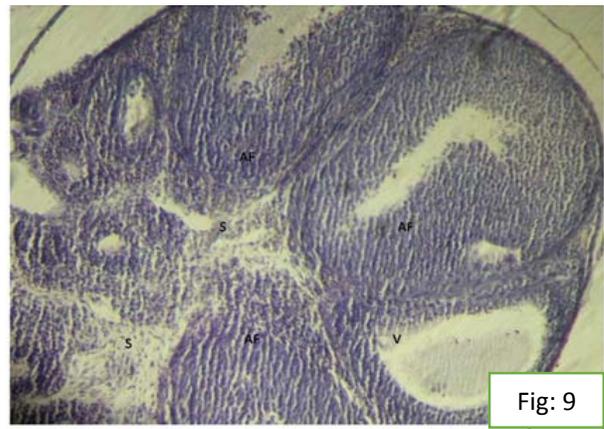


Fig: 9

Fig 9: Photomicrograph of paraffin section of experimental ovary of rat, *R. norvegicus*, fed with contaminated fish meal diet for 30 days showing plenty of unhealthy follicles and irregular germinal epithelium

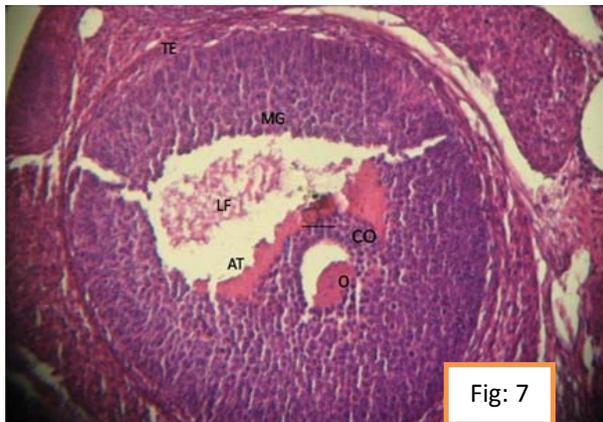


Fig: 7

Fig 7: Photomicrograph of paraffin section of control ovary of rat, *R. norvegicus*, showing early graafian follicle with growing antrum filled with liquor folliculi. Theca externa shows well organised intact spindle shaped cells

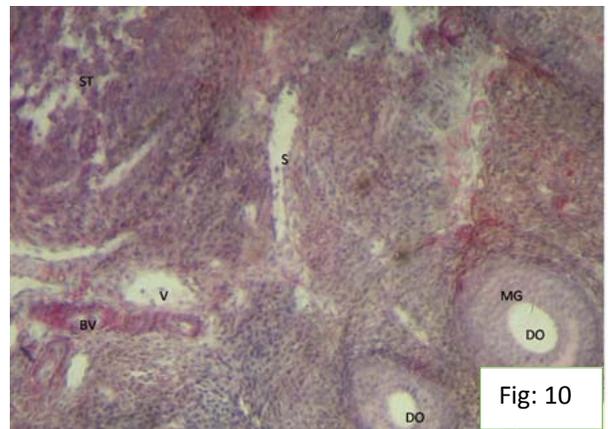


Fig: 10

Fig 10: Photomicrograph of paraffin section of experimental ovary of rat, *R. norvegicus*, fed with contaminated fish meal diet for 30 days showing follicle with dissolved oocyte and increased spaces and vacuoles among interstitial cells

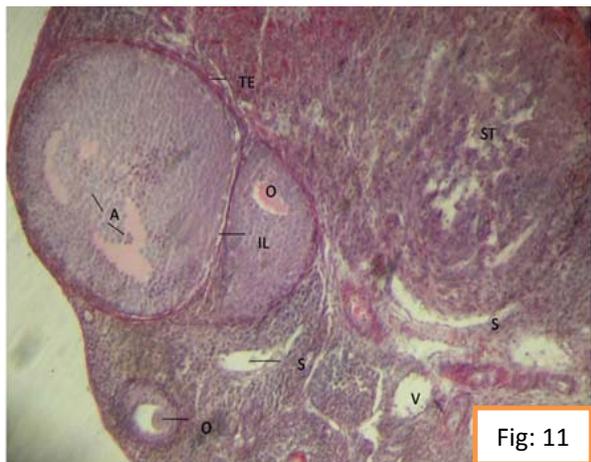


Fig: 11

Fig 11: Photomicrograph of paraffin section of experimental ovary of rat, *Rattus norvegicus*, fed with contaminated fish meal diet for 30 days showing follicle with secondary follicle showing formation of theca externa between oocyte and disorganised antrum with dilated blood vessel

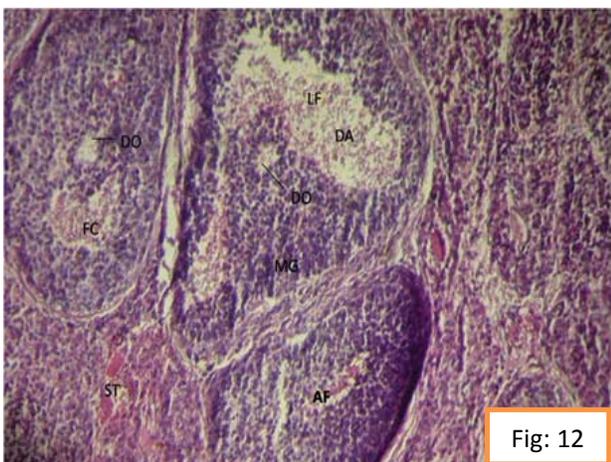


Fig: 12

Fig 12: Photomicrograph of paraffin section of experimental ovary of rat, *Rattus norvegicus*, fed with contaminated fish meal diet for 30 days graafian follicle with disintegrated zona pellucida and distorted oocyte.

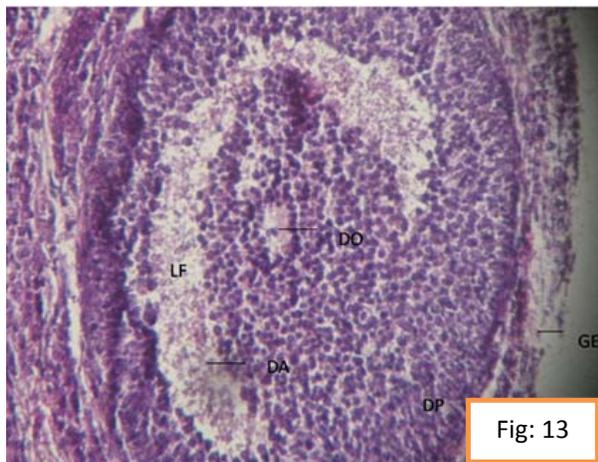


Fig: 13

Fig 13: Photomicrograph of paraffin section of experimental ovary of rat, *R. norvegicus*, fed with contaminated fish meal diet for 30 days graafian follicle with irregular shaped disintegrated antrum.

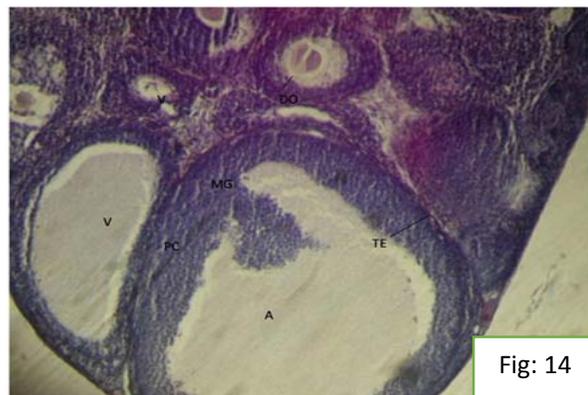


Fig: 14

Fig 14: Photomicrograph of paraffin section of experimental ovary of rat, *R. norvegicus*, fed with contaminated fish meal diet for 30 days graafian follicle with distorted oocyte and absence of liquor folliculi in the antrum and membrana granulosa shows pycnosis.

Abbreviations

DF - Developing Follicle, AF - Atretic Follicle, ST – Stroma, ZP - Zona Pellucida, V - Vacuole, O- Oocyte, LF - Liquor Folliculi, PF - Primary Follicle, SF - Secondary Follicle, CO - Cumulus Oophorus, GE - Germinal Epithelium, BV - Blood Vessel, MG - Membrana Granulosa, TE - Theca Externa, MV - Microvilli, DA - Developing Atrum, DP - Discus Proligerous, GF - Graafian Follicle, DO - Dissoluted Oocyte, IL - Intermediate Layer, S – Space, NC - Nucleated Cells

Discussion and Conclusion

Eldridge *et al.*, (1994)^[5] and Gojmerac *et al.*, (1996)^[7] have reported pesticides to decrease estrogen levels, which in turn suppress estradiol concentration in blood and reduce the production of estradiol by ovarian cells. Histological studies were made to find out the effect of contaminated fishmeal stress on the rat ovary. Results revealed that rat fed with contaminated fish meal for 7 days caused increase in number of atretic follicles. However, after 30 days treatment it is assumed that the contaminated fish meal inhibits oogenesis and the granulosa cells showed apoptosis, which resulted in loss of the functional activity as evident from failure of the follicle cells to synthesize ovarian hormones. There was delay in the estrous cycle and irregular duration of each phase particularly in diestrous phase. These results show contaminated fish meal to be exerting various toxic effects upon steroid hormones. Similar results have also been noticed in rats treated with pesticides (Adilaxmamma *et al.*, 1994)^[1] Ratnasooriya *et al.*, (1995)^[13] are of the opinion that, decrease in weight and size of ovaries may be due to extensive fibrosis and atretic follicle formation. While are of the opinion that Monocrotophos in higher doses suppresses food and water intake and thereby decreases body weight in fishes. Plowchalk *et al.*, (1993)^[11] have opined that a quantitative assessment of follicle number can be used an indicator of the normal function as well as toxic responses of the ovary. Although all follicles are apparently exposed to the same fluctuations in hormones, not all are equally responsive, some ovulate and others become atretic, indicating the presence of intragonadal regulatory factors which modulate the effects of these hormones (Dorrington *et al.*; 1988)^[6]. The present study revealed healthy follicles to significantly decrease with significant increase in atretic follicles in treated rats. Similar findings have been reported in rats treated with different pesticides Jadaramkunti and Kaliwal 1999^[10].

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